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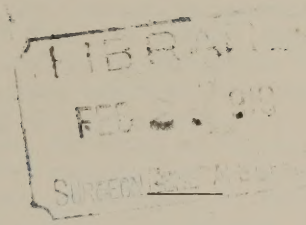
PRELIMINARY REPORT



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PROPHYLACTIC INOCULATION AGAINST RESPIRATORY INFECTIONS

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In attempting to lessen the incidence and to reduce the severity of infections of the respiratory tract by vaccination, it is essential to consider the wide range of bacterial flora, the relative prevalence of each species, as well as the fluctuations in incidence and severity of these infections with changes in season. The well-defined tendency of bacteria of the same species to localize differently in different epidemics indicates peculiar infecting and antigenic powers. The short duration of immunity to infections following attacks adds greatly to the difficulty. However, owing to the high incidence and high mortality rate from infections of the respiratory tract during the present epidemic, a painstaking effort to raise the resistance of individuals by inoculation with appropriate vaccines appeared to be strongly indicated.

In considering prophylactic inoculations in this epidemic of influenza, we put aside the debated question as to the cause of the initial symptoms and considered primarily the possibility of immunizing persons against the bacteria, pneumonia, streptococci, influenza bacillus and staphylococci, which are conceded by all to be the common causes of death in this disease. It was thought that it might be possible to raise to some degree, by artificial means, the immunity of persons to these micro-organisms to which they appear so susceptible, and thus to lower the incidence of the more serious respiratory infections, particularly pneumonia.

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* From the Mayo Foundation.
* Read before the American Public Health Association, Chicago, Dec. 10, 1918.

The bacteria found as the cause of the complications in this epidemic appear to have exalted and peculiar infecting powers. The mode of death and the findings in the lungs, for example, in the so-called acute bronchopneumonia following influenza, are quite unique and are strikingly similar, irrespective of the species of micro-organism present. Infection of the lung by hemolytic streptococci without empyema and without tonsillitis indicates peculiar localizing power of this micro-organism. The influenza bacillus appears to have acquired peculiar virulence. The frequency of staphylococci in the sputum and lung associated with pneumococci and streptococci far exceeds that which occurs in lobar pneumonia. A study of the various strains isolated has revealed commonly marked variations in cultural and other properties. Owing to these findings, it was the plan to prepare the vaccine not from saprophytized laboratory strains, as is too often the rule in vaccine therapy, but from strains freshly isolated from the sputum and lungs, and to incorporate the bacteria in the vaccine in about the proportion in which they are found, and before the more or less peculiar properties disappear. A bacteriologic study, made during the progress of the epidemic, showed a decided change in the bacterial flora, and hence new strains were added from time to time in order that the vaccine might represent as nearly as possible the bacterial flora of the disease at various stages of the epidemic.

Heretofore lobar pneumonia has been unusually prevalent for some months following epidemics of influenza. It was felt that this would be particularly apt to be the case following the present epidemic, since it began early in the season. It was decided, therefore, to include a series of the fixed types of pneumococci in the vaccine, although they were infrequently isolated, especially during the early part of the epidemic.

A study of the secretions from the nose and throat, of the sputum and lung exudate from the very beginning of the epidemic as it occurred in and about Rochester, revealed commonly, among other bacteria, a streptococcus having some distinctive features. Smears from the nose and throat and sputum at the onset of the attack show quite constantly large numbers of this organism in the form of gram-positive lanceolate

diplococci occurring singly, but more often in rather long chains. The epithelial cells are frequently found packed with this micro-organism. On artificial cultivation of these exudates it presents morphologic and cultural features both of the pneumococcus and of *Streptococcus viridans*. It produces on isolation a rather moist, spreading, nonadherent, greenish colony on blood agar plates, and a diffuse cloud in glucose broth. On solid mediums it grows as a lanceolate diplococcus of quite uniform size, and usually is surrounded by a distinct capsule. In glucose broth it produces lancet-shaped diplococci in rather long chains. Smears from the older cultures often show extreme variations in size and shape. Injection of sputum into guinea-pigs is usually followed by death from peritonitis, the peritoneal exudate and blood showing this organism in pure or almost pure form. It is more virulent than the green-producing streptococci from the throats of normal persons, but is less virulent than pneumococci from lobar pneumonia. The strains do not usually ferment inulin. It does not autolyze readily and is not soluble in bile. Owing to these findings the vaccine was made to contain a heavy mixture of these strains.

The organism is undoubtedly the one found by the English investigators and designated by them as diplostreptococcus, and the one found by Mathers and for which Tunicliff finds an increased opsonic content in the serum of convalescent patients. Immunologic and other studies to determine further the relations of this organism to influenza are under way and will be reported later.

Hemolytic streptococci were found next most frequently, particularly in fatal cases during the first half of the epidemic. *Staphylococcus aureus* and influenza bacilli appear to play a minor but definite rôle in the production of the complicating pneumonia in some cases. Hence, examples of strains of hemolytic streptococci, staphylococci and influenza bacilli were included in the vaccine.

PREPARATION OF THE VACCINE

The formula of the vaccine used during the earlier part of the epidemic, and exclusively in the cases in this report, is given in Table 1. The bacteria were

grown for from eighteen to thirty-six hours at from 33 to 35 C., in 0.2 per cent. glucose broth. The broth was autoclaved at 20 pounds pressure for from one to two hours to insure freedom from living spores. The glucose was added in a sterile manner from a concentrated sterilized solution in water. It was found

TABLE 1.—FORMULA OF VACCINE

Pneumococci, Types I (10 per cent.), II (14 per cent.) and III (6 per cent.)	30 per cent.
Pneumococci Group IV and the allied green-producing diplostreptococci described	30 per cent.
Hemolytic streptococci	20 per cent.
Staphylococcus aureus	10 per cent.
Influenza bacillus	10 per cent.

that the cultures of pneumococci and streptococci yielded approximately 1,000 million bacteria, and *Staphylococcus aureus* 2,000 million bacteria per cubic centimeter. Luxuriant growth (about 1,000 million per cubic centimeter) of the influenza bacillus was obtained by adding approximately 1 c.c. of laked human blood per liter of glucose broth. The strains were grown separately in the flasks. Smears were made before centrifugation of each flask to eliminate possible contaminations. The pneumococci and allied green-producing streptococci, the staphylococci and influenza bacilli were separated from the broth culture by centrifugation,[1] and then suspended in sodium chlorid solution. At first 50 per cent. of the hemolytic streptococci were added in the form of the killed broth culture, and the other 50 per cent. in sodium chlorid solution suspension after centrifugation. But owing to rather severe reactions, only 25 per cent. of the hemolytic streptococci are added in the form of the broth culture. The streptococci in the broth culture

1. In connection with some work on poliomyelitis in which it became necessary to procure large quantities of the streptococcus, the ordinary large cup centrifuge proved inadequate. A number of centrifugal machines were tested to see if they might not facilitate greatly the clarification of broth in its preparation and to separate efficiently the bacteria from large quantities of liquid cultures. A number were found useful; but owing to the simplicity of construction and the ease with which bacteria may be obtained from the revolving bowl without contamination, the one manufactured by the Sharples Separator Company, West Chester, Pa., was selected and has proved satisfactory for the purpose. By the use of the small laboratory size, it is possible, for example, to separate the bacteria from 50 liters of broth an hour. The revolving bowl and other utensils with which the broth comes in contact are autoclaved. A galvanized iron hood built over the machine makes it possible to sterilize the air in the hood with steam; and by siphoning the broth cultures from the bottles, large quantities of bacteria may be collected without contamination.

are killed by the addition of 0.5 per cent. cresol. The centrifugated bacteria are suspended in sodium chlorid solution so that 1 c.c. represents approximately the growth from 50 c.c. of the broth culture, and are killed by the addition of from 1 to 1.5 per cent. purified cresol. The dense suspensions are diluted with an equal volume of sodium chlorid solution after the cultures, made twenty-four hours after the cresol is added, have remained sterile for forty-eight hours. In some instances the suspensions became slightly contaminated with *Bacillus subtilis,* when heating to 60 C. for one hour was necessary to render them completely sterile. If this was not sufficient, the suspensions were discarded. At first, owing to the urgent demand for the vaccine, the use of extreme heat in the sterilization of the broth, negative aerobic and anaerobic cultures at the end of from forty-eight to seventy-two hours were considered sufficient as sterility tests. It is now the rule to hold the vaccine until all cultures and animal tests have proved negative for one week. Blood agar and glucose broth and glucose brain broth and litmus milk in tall columns are the mediums used for the sterility tests. The vaccine is finally made up by diluting the dense suspensions in the proper proportions with sodium chlorid solution so that 1 c.c. contains approximately 5 billion bacteria. Purified cresol (0.3 per cent.) is used as a preservative. The initial dose for adults is 0.5 c.c. subcutaneously. This is followed in one week by 1 c.c. The third dose is given fourteen days after the first dose, and consists of 1.5 c.c. The doses in children are 0.1, 0.2 and 0.3 c.c. or more according to age. Owing to the relatively large doses, intervals shorter than one week between injections are considered inadvisable.

RESULTS

In most instances there is a moderate local reaction. Constitutional symptoms are usually mild or absent. Both local and general reactions are decidedly less than those following typhoid vaccines. Exceptionally severe reactions occur showing unusual individual susceptibility. The severer reactions are prone to occur in persons who give a history of recent exposure to the disease, or who already have beginning symptoms of it. In these persons, attacks may appear to be precipitated by the vaccination; but the course

is usually short and relatively mild. Persons appear to be more sensitive to the vaccine during the incubation period than before or after the attack is established, or following recovery.

Physicians, nurses and employees of the several hospitals in Rochester were first inoculated. After it was determined that injections of the vaccine were followed by an increase in antibodies in the serum, that the inoculations appeared to be quite harmless, that there was no increased incidence of respiratory infection following the inoculations but an apparent decrease instead, it was decided to study its effects on

TABLE 2.—INCIDENCE OF ILLNESS AND MORTALITY FOR ONE THOUSAND PERSONS

	Inoculated					Uninoculated
	After First Inoculation	After Second Inoculation	Within 7 Days after Third Inoculation	Within 6 Weeks after Third Inoculation	Total	
Influenza	23	10	9	14.6	56.6	229
Pneumonia	1.8	1	1	1.8	5.6	15.7
Meningitis	0	0	0	0	0	0.4
Encephalitis	0.04	0	0.05	0	0.09	0.2
Deaths from influenza or pneumonia	0.63	0.08	0	0.19	0.9	3.4
Total number of inoculated and uninoculated persons	28,459	26,150	20,792	20,792	....	61,753

a larger scale as the epidemic became acute. The vaccine was sent gratis for study by the Mayo Foundation to hospitals and physicians on request, on condition that reports of results be furnished.

The reports included in this paper are from physicians, hospitals and other institutions, chiefly from cities and towns within a radius of 200 miles from Rochester.

In Table 2 is given a summary of the results obtained in a considerable number of inoculated and uninoculated control persons. The reports containing results in the vaccinated after the epidemic was on the decline are not included. The uninoculated represent such persons in institutions, colleges, factories, corporations and communities where the vaccine was used. Only those reports are included that contain

accurate data as to the incidence and mortality among the uninoculated. The age in both the inoculated and the uninoculated falls mainly between twenty and forty years. The observations were made from Oct. 15 to Dec. 8, 1918. It will be seen that there was no apparent negative phase following the first inoculation and that there was a progressive diminution of incidence and death from influenza and pneumonia following the successive inoculations. The total incidence of recognizable influenza, pneumonia and encephalitis in the inoculated is approximately one-third as great as in the control uninoculated. The total death rate from influenza or pneumonia is only one-fourth as great in the inoculated as in the uninoculated. No cases of meningitis occurred in the former, whereas in the latter there were 0.4 per thousand. These results were obtained by including all persons who developed influenza or pneumonia and all who died from the day of the first inoculation, and 7,667 persons, or about one fourth of the total number who received only one inoculation.

The total incidence and death rate in the uninoculated controls are well within the average, as they occur during the present epidemic and hence serve as a fair basis for comparison. Experiments in which alternate control persons were inoculated were not done because of difficulty to obtain consent, and because, after all, the results from prophylactic inoculation must be sufficiently favorable to be apparent under the conditions included in this report.

All but two of over seventy physicians who have used the vaccine report that the attacks of influenza, if contracted by the inoculated, are milder and of shorter duration, and that convalescence is more rapid than in the uninoculated. This agrees with our observations, and is in keeping with the lower death rate among the inoculated. This difference has been noted in communities in which the incidence and mortality rate were exceptionally high as well as where they were comparatively low. In view of this observation, granting that the initial symptoms in influenza may be due to an unknown virus, the lowered incidence of influenza among the inoculated may be only apparent. The attacks may have been so mild as to escape detection.

Among the nurses at St. Mary's Hospital, Rochester, where fourteen developed influenza within two days prior to the first inoculation, only one case developed subsequently during a period of six weeks. Similar apparent protection was afforded to the personnel of other hospitals following vaccination. At the State Hospital for the Insane at Rochester, with a total population of about 1,500, where one case of influenza had occurred before the inoculations were given, only three cases occurred following the date of the first inoculation for a period of six weeks. With the occurrence of the second wave of the epidemic, however, there occurred a mild outbreak of the disease. This would indicate that the immunity is of short duration.

Nearly all of the patients with influenza and pneumonia admitted for treatment in the hospitals in Rochester, where approximately one half of the population has been vaccinated, have been from the uninoculated group, excluding those patients who contracted the disease elsewhere.

In one hospital in which the nurses had been inoculated, no cases developed after the inoculations, although the nurses continued to care for patients with influenza. Owing to the scarcity of vaccine, some of the nurses, living under identical conditions, were not inoculated, and a high percentage of these contracted severe attacks.

Numerous instances have been observed in which protection appeared to be afforded to inoculated members of families of which all the uninoculated became ill. Similar results were obtained when conditions among the inoculated and uninoculated were comparable, such as in offices, factories and schools, where nearly all were inoculated, or where only a small percentage were inoculated. Illustrating results are as follows:

Of 1,000 persons employed by one company, 481, about one half, received one inoculation; 224 received two inoculations, and ninety-five received three inoculations. From October 28, the date of the first inoculation, to December 8, 138 cases of influenza occurred, only twenty of which were among persons who had had one or more inoculations. Of these, fourteen had had only one inoculation, and the remaining six had but two inoculations. There were thirteen deaths, only two of which followed influenza among the inocu-

lated, and in these two cases only one inoculation had been given.

The mortality from bronchopneumonia in pregnant women has been especially high during the present epidemic. The vaccinations in a fairly large number of such persons appear to have afforded some protection against this complication. The bacteria included in the vaccine belong to the general group of microorganisms associated commonly with chronic infections, such as arthritis, sinusitis and bronchitis; hence some effect should follow its injection. Striking instances of improvement in these conditions have been noted but whether due to specific or nonspecific effects or whether the vaccine acts as an "exfoliative stimulus" according to Larson, liberating preformed specific antibodies, remains to be determined.

From the results obtained thus far, it appears possible to afford a definite degree of protection by prophylactic inoculation to persons against the more serious respiratory infections during the present epidemic of influenza. The duration of immunity is not known, but indications are that it is relatively short.

The vaccine should contain freshly isolated strains of the more important bacteria in approximately the proportions as found in the sputum and lungs in the disease, and since the relative proportions of the bacteria at hand differ so markedly in widely separated communities, judging by the reports, the formula of the vaccine should be made to conform as nearly as practicable to the respective flora of the disease in the communities in which the vaccine is to be used.

A saline vaccine was used as an emergency measure. Owing to the large number of different bacteria that need to be included and the large doses necessary, a lipovaccine, judging by the recent work of Whitmore, ought to possess definite advantages, since reactions should be less severe, the formation of antibodies more marked, and the resulting immunity more enduring.

I am constantly being asked with regard to the use of the vaccine in treatment. Since the severer complications in influenza, such as pneumonia, do not usually begin until the fourth day or later, the vaccine, if given at the onset of the disease might reasonably be expected to afford some protection. The initial

prophylactic dose daily for one, two or three days, provided no unfavorable symptoms occur, is recommended. The results obtained are considered preliminary, and final conclusions cannot be drawn at this time. It is indicated that the vaccine used was at least harmless, that a certain degree of protection was afforded, and that prophylactic inoculation against the respiratory infections, so fatal during this epidemic, be studied on a large scale by many according to the principles herein laid down.

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